

**ProFoldin**

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INSTRUCTIONS

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E. coli D-Alanine :D-Alanine Ligase

E. coli* D-Alanine: D-Alanine Ligase – for 100 assays*Catalog No. DDL-100EC**

Protein construct: Wild-type *E. coli* D-Alanine: D-Alanine Ligase purified from a bacterial expression system.

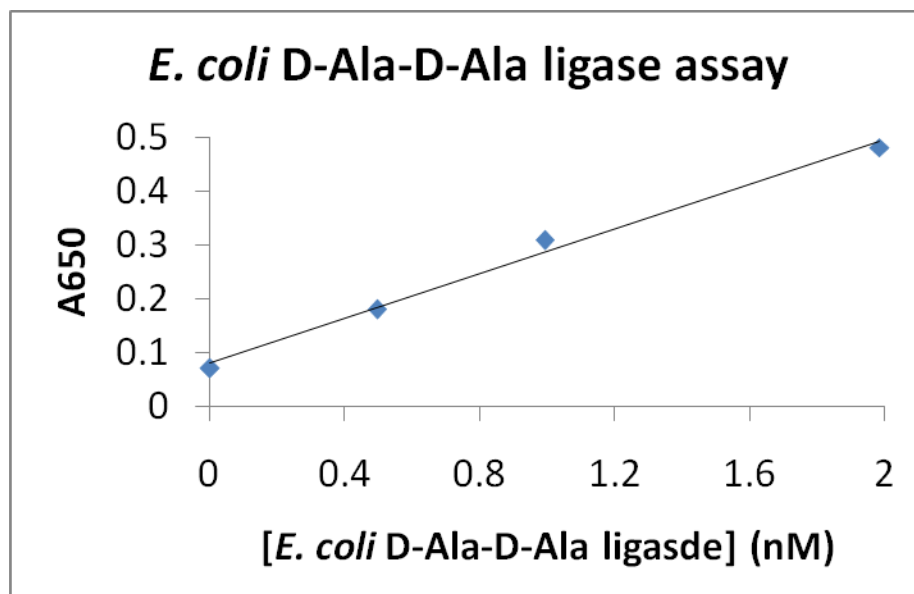
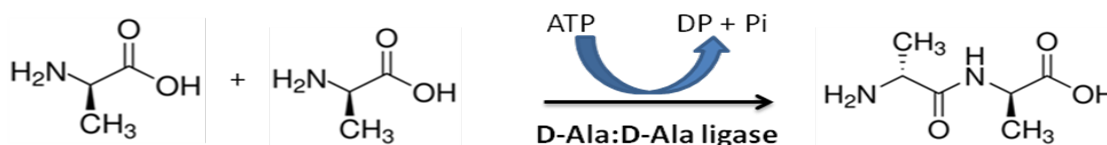
MW: 39 kDa

Enzyme concentration: 200 nM

Enzyme activity assay: The D-Alanine: D-Alanine Ligase activity is measured by using the *E. coli* D-Alanine: D-Alanine Ligase Assay Kit (Catalog No. DDA100K).

Storage temperature: -20 or -80°C. Do not freeze-and-thaw repeatedly.

Enzyme dilution: Use the 1 x assay to dilute the enzyme just before the assay. Do not store diluted enzyme solution



The *E. coli* D-Alanine: D-Alanine Ligase Assay – for 100 assays (Catalog No. DDL-100EC) contains 33 µl of 100 x *E. coli* D-Ala:D-Ala ligase (200 nM). It is for 100 assays.

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Assay protocol using the *E. coli* D-alanine: D-alanine ligase assay kit

The following assay protocol is based on the 384-well plate assay format. The reaction volume is 30 µl and the final assay volume is 75 µl. For 96-well plate assays, the reaction volume is 60 µl and the final assay volume is 150 µl. For detection using a cuvette, the reaction volume is 400 µl and the final assay volume is 1000 µl.

1. Reagent preparation:

For each 10 assay reactions,

- (1) Prepare 297 µl of premix composed of 261 µl of H₂O, 33 µl of 10 x Buffer and 3.3 µl of 100 x *E. coli* D-Ala:D-Ala ligase.
- (2) Prepare 33 µl of 10 x Enzyme substrate by mixing 3.3 µl of 100 x Enzyme substrate with 29.7 µl of water.

2. Reaction:

Mix 27 µl of the premix with 3 µl of the 10 x Enzyme substrate in each well. Incubate the reaction mixture at 37°C for 60 min.

3. Detection:

Add 45 µl of the Dye MPA3000 into the 30 µl of the reaction mixture. Incubate for 5 min. Measure the light absorbance at 650 nm.

Assay protocol for enzyme inhibition

The assay can be optimized in terms of assay window, assay linearity and sensitivity to competitive inhibitors. ProFoldin offers HTS assay development service. For more information, please visit our website at <http://www.profoldin.com/services.html>.

Related Products

<i>E. coli</i> D-alanine D-alanine ligase Assay Kit	DDA100K
<i>E. coli</i> D-alanine D-alanine ligase Assay Kit Plus-100	DDA100KE
<i>E. coli</i> MurA Assay Kit Plus-100	MURA100KE
<i>E. coli</i> MurC Assay Kit Plus-100	MURC100KE
<i>E. coli</i> MurD Assay Kit Plus-100	MURD100KE
<i>E. coli</i> MurE Assay Kit Plus-100	MURE100KE
<i>E. coli</i> MurF Assay Kit Plus-100	MURF100KE
<i>E. coli</i> GlmU Assay Kit Plus-100	GLU100KE

More information of drug targets and enzyme assays

For more information of drug targets and enzyme assays, please visit www.profoldin.com or send emails to info@profoldin.com.